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## Abstract

*Adult male rats produce 22-kHz vocalizations when exposed to a female conspecific. These ultrasonic vocalizations (USV) are a measure of the male's level of sexual arousal. Previous studies have shown that antipyretic drugs diminish the amount of USV made by male rats. Male mice also emit USV (70- kHz) indicative of sexual arousal. In this study, the effects of sodium salicylate, an antipyretic drug, were examined to see if USV were diminished in mice. Fifteen male adult mice were tested in a Treatment (Saline vs. Sodium Salicylate) x Dose (High vs. Low) design, with repeated measures across the treatments. Vocalizations and latency to mount a female were used as dependent measures. The results indicated that at either dose sodium salicylate diminished USV and increased mount latencies. Futhermore, animals in the high dose groups took longer than animals in the low dose group to mount females following treatment with saline injections. These findings suggest that antipyretics, such as sodium salicylate, may diminish sexual arousal in mice and rats.*

Ultrasonic vocalizations (USV) are emitted by rodents in response to a variety of stimuli (Nyby & Whitney, 1978; Sales & Pye, 1974). Many rodents, from lemmings to hamsters and rats to mice, emanate these USV. All of these vocalizations seem to be made in the social contexts of interactions with other conspecifics. Infant rodent vocalizations tend to be made when pups are isolated from the dam and littermates. These infant distress vocalizations help the dam locate and retrieve the pup. Adult rodents tend to vocalize during aggression and copulatory behaviors. Thus, adult and infant USV appear to be correlated with periods of high arousal. Although much is known about the stimulus that elicit vocalization, relatively little is known about their neurochemical regulation (Miczek, Tornatzky, & Vivian, 1991).

Conely and Bell (1977) found that when isolated from the dam and littermates, neonatal rats emitted a higher number of vocalizations when female odor cues were used as stimuli. When odors from adult males were used as the stimulus, fewer vocalizations were produced. A similar study by Oswalt and Meier (1975) tested neonatal rats exposed to no bedding, clean bedding, and soiled bedding from the home cage. They found significantly more vocalizations were produced in the no bedding and clean bedding situations as compared to the soiled bedding situation. The greater the presence of elements of the home cage, or the more familiar the environment to the subject, the fewer vocalizations were produced.

Hofer, Shair, and Murowchick (1989) performed a study comparing the ultrasonic vocalizations of isolation reared pups to those of pups reared in natural litters. Results showed that isolation reared pups vocalized significantly more than pups reared in their normal nest environment. The test also revealed that isolation reared pups were significantly more motile and produced more rising motions than control pups.

Infant rats vocalize primarily in response to isolation from the dam, but falling body temperature also affects vocalizations (Blumberg, Efimova, & Alberts, 1992a, 1992b). Allin and Banks (1971) showed that as ambient temperature dropped below the pups body temperature, vocalizations increased dramatically through postnatal day 14. Other investigators have suggested that the onset of infant vocalizations within cold enviroments is correlated with activation of metabolically active tissues (i.e. brown adipose tissue) used to generate heat (Blumberg & Alberts, 1990).

Hofer and Shair (1992) suggest that infant rats emit USVs in response to

isolation, handling, novelty, and cold. They note that the ability of mice to thermoregulate develops through the first two weeks after birth. During the first week of life, body temperature is largely dependent upon the temperature of the external environment (i.e., the mice are poikilothermic). During the second week of life, the ability of the mice to thermoregulate continues to develop (i.e., they become homeothermic). Hofer and Shair suggest that during the second week, isolation becomes the primary reason for vocalizations. This study showed changes in the underlying causes for vocalization within the first two weeks after birth. In the first week of life, vocalizations were caused by falling body temperature, and in the second week of life, vocalizations were caused by anxiety. This conclusion is supported by data showing that during the second week, infant USVs are reduced following treatment with a variety of anxiolytic drugs (Benton & Nastiti, 1988). Opiates also decrease infant USVs, while opiate antagonists, e.g., naloxone, have been found to increase vocalization (Herman & Panksepp, 1978).

In one of the first studies to examine the neurochemical mediation of infant ultrasonic vocalizations, Herman and Panksepp (1978) found that morphine, in dose dependent fashion, significantly reduced the amount of isolation calls made by infant gerbils. In their study, treatment with the opiate antagonist naloxone increased the number of vocalizations that were emitted by isolated infants. The apparent anxiolytic effects of opiates on infant vocalizations has been replicated numerous times (Kehoe & Blass, 1986).

Carden and Hofer (1990b) found that rat pups generally cease to vocalize roughly 5-10 minutes after being isolated. They attribute this to a release of endogenous opiates. A study with newborn rats in isolated conditions inferred that as anxiety levels increase, and a certain elevated level of anxiety is attained, an internal system of stress relievers (endogenous opiates) are released. These bind to opiate receptors, and consequently, reduce sensitivity to pain and ease anxiety (Blass & Kehoe, 1987).

Morphine, a potent opiate agonist, acts on opiate receptors to suppress the anxiety. In another study, Carden and Hofer (1990a) produced similar effects of morphine on infant rat vocalizations. In addition, they tested chlordiazepoxide (CDP), a benzodiazepine (BDZ), and found that both CDP and morphine had similar anxiolytic effects. They also tested a naltrexone (NLX) condition. Naltrexone, an opiate antagonist with pharmacological properties similar to naloxone, significantly increased vocalizations in rat pups. Apparently NLX prevented endogenous opiates from binding to opiate receptors, thereby maintaining, and even elevating rates of vocalization. Then Carden and Hofer introduced several drug conditions to determine if the pathway of NLX and CDP was the same. Results indicated the independence of the NLX pathway from the BDZ pathway and found that indeed the NLX pathway influences endogenous opiate receptors independent of the BDZ pathway. This experiment shows that pharmacological regulation of USV works in more than one way.

Like infants, adult rodents emit ultrasonic vocalizations in a number of social contexts. One such context is courtship and reproduction (Nyby & Whitney, 1978). In these contexts male mice emit 70-kHz vocalizations in response to females or their odor (Nyby, 1983; Whitney, Alpern, Dizinno & Horrowitz, 1974). Dominant male mice tend to vocalize more than subordinate males. This difference may be due to higher levels of testosterone in the dominant males. High rates of male vocalization are apparently attractive to females (Pomerantz, Nunez, & Bean, 1983).

Adult male rats also emit USV during sexual interactions with receptive females (Nyby & Whitney, 1978). In particular, males emit 22-kHz vocalizations following ejaculation. Blumberg and Alberts (1991) concluded that these post-copulatory vocalizations are caused primarily by an increased

hypothalamic temperature. Blumberg and Moltz (1987) have suggested that adult vocalizations may help cool the hypothalamus by facilitating the flow of the nasal venous blood entering and leaving the hypothalamus. They found a rapid decrease in temperature of the hypothalamus during the post-ejaculatory period, that corresponded with the onset of post-ejaculatory USV in male rats.

In related experiments, Blumberg and Moltz (1987) manipulated the temperature of the hypothalamus by infusing prostaglandin $E_2$ ($PGE_2$) into the hypothalamus to elevate the temperature, or by systematically injecting sodium salicylate to lower hypothalamic temperature. Results showed that when infused with $PGE_2$, temperature rose and vocalizations generally increased, but not significantly enough to conclude that hypothalamic temperature is the only variable influencing vocalizations. In contrast, sodium salicylate significantly lowered mean hypothalamic temperatures and diminished post-ejaculatory vocalizations, presumably due to the drug's antipyretic agents.

This paper summarizes a study in which the effects of sodium salicylate on adult male mouse sexual arousal were observed. This was the first study to test Blumberg and Moltz's (1987) hypothesis in mice. Also, in addition to monitoring USV, the male's latency to mount a female was included as another gauge of male sexual arousal. If the function of USV is to regulate hypothalamic temperature, we expected to find fewer vocalizations and longer mount latencies in male mice treated with sodium salicylate. The antipyretic actions of the drug should lower the temperature of the hypothalamus, decrease sex arousal, reduce the number of vocalizations , and lengthen mount latency. It is also possible that sodium salicylate may lower brain temperature, thereby decreasing USV, but may not affect other indicators of sexual arousal (i.e., mount latency).

## Method

### *Subjects*

The subjects were 15 male $F_1$ offspring of male AKR and female C57BL/6 mice obtained from Charles River Laboratories (Wilmington, MA). All mice were born and raised in plastic mouse terraria (48 x 26 x 13.5 cm). At 21 days of age, all mice were individually housed in temperature and humidity controlled rooms on a reversed 12:12 hr light:dark cycle (lights on at 1800 hours). All subjects received social experience with a female conspecific for 3 min each day for eight days. Adult (> 50 days of age) male AKR mice were also used for socialization to establish dominance traits for the subject males.

The order of male/female social experience was counterbalanced across days. Adult female C57BL/6 mice were the stimulus urine donors for the experiment. The stimulus urine donor females were placed in metabolic cages 24 hours prior to the experiment to collect urine. The subjects ranged in age from 130 to 180 days of age on the first day of testing.

### *Materials*

A bat detector (Model S-25, Ultrasound Advice; London, England), was used to convert the USV into audible signals. A microphone was suspended 10 cm above the subject's home cage and transmitted the sounds to the bat detector. Sodium salicylate (Merck) injections were administered intraperitoneally (ip) at doses of either 150 mg/kg or 75 mg/kg. Each subject was also tested following controlled injections of saline (0.9%).

### *Procedure*

The experiment involved two groups: high dose (150 mg/kg), and low dose (75 mg/kg). All subjects were screened by testing vocalizations, in the presence of a female, over a three minute

period. Subjects in each group were tested following treatment with sodium salicylate and saline. The order of injections were counterbalanced (half of each group receiving saline on the first test day and the other half receiving the drug treatment). Each group had 48 hours between tests in the counterbalanced condition. Thirty minutes prior to the test, each male was administered an ip injection of the proper dosage of the drug.

Just prior to testing, each subject was placed into a clean home cage with unsoiled bedding, and a one minute period of habituation ensued. If the animal vocalized during the habituation period, then the experiment did not continue until at least two minutes of nonvocalizing occurred. None of the subjects vocalized during the habituation period.

Following habituation, a cotton swab with 0.1 ml female urine was placed in the subject's cage and vocalizations were recorded for three minutes. At the end of the three minutes, a female conspecific was placed into the cage and vocalizations and mount latency were monitored for two minutes. At the conclusion of the test period, the stimulus female was removed, and the subject was returned to the colony.USV were quantified by counting the number of 5 s bins in which the experimenter detected the presence of vocalizations. The maximum USV score that could be obtained in response to female urine was 36. The maximum possible USV score in response to the female was 24. Throughout the experiment, the person monitoring vocalizations was unaware of the type of treatment each subject had been given.

## Results

A 2 (treatment) x 2 (dose) ANOVA indicated that male USV in response to female urine was significantly less following treatment with sodium salicylate than treatment with saline, $F$ (1,13) = 12.40, $p < .01$. The mean ± $SE$ USV response to urine following treatment with sodium salicylate was 0.94 ± 0.67, while that obtained following saline treatment was 6.85 ± 1.66. There was no significant interaction between dose and treatment.

A similar pattern of results was obtained when the male was tested with a female conspecific. Following treatment with saline or with sodium salicylate, the mean USV response to the female was 18.03 ± 1.03 and 9.91 ± 1.51 respectively, $F$ (1,13) = 12.52, $p < .01$. Once again there was no interaction between the treatment and dose effects.

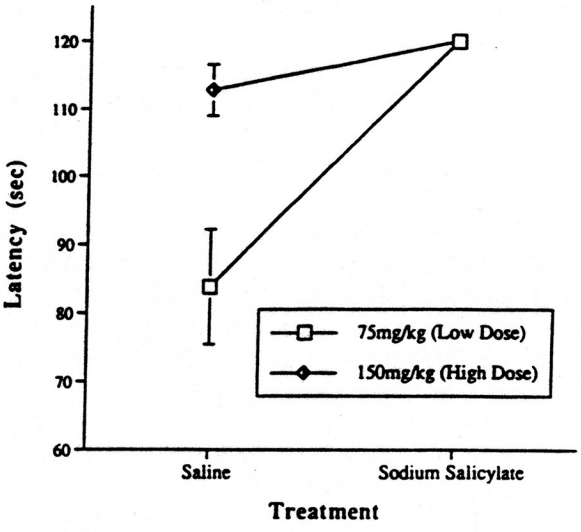


*Figure 1.* Mean ± $SE$ mount latency of male mice in the low and high dose groups, following treatment with saline and with sodium salicylate. After 2 minutes (120 s) the trial was terminated.

Males treated with sodium salicylate took significantly longer to mount the female than males given saline, $F$ (1,13) = 22.27, $p < .001$. In the drug condition, the mean ± $SE$ mount latency (s) was 118.39 ± 1.21, and in the saline condition it was 95.45 ± 4.19. In comparing the effects of the low and high dose treatments, the ANOVA indicated a significantly longer mount latency in the 150 mg/kg group than the 75 mg/kg group, $F$ (1,13) =10.76, $p < .006$. The means ± $SE$ for animals in the low dose and the high dose group were 101.93 ± 6.43 and 116.63 ± 2.07, respectively. There also was a significant interaction effect between dose and

treatment conditions, $F$ (1,13) = 10.76, $p <$ .006.

Figure 1 summarizes the mean latency to mount the females in both low and high dose groups following treatment with sodium salicylate and saline. Following treatment with sodium salicylate, the average mount latency was the same regardless of the group the animal was in, i.e., high versus low. However, animals in the high dose condition had longer mount latencies in the saline condition, than did animals in the low dose condition.

## Discussion

The results of this study support previous findings that male mice emit more vocalizations in response to females than to female urine alone (Nyby & Whitney, 1978). It was possible for male mice in this experiment to obtain a raw USV score of 36 in response to female urine alone and a maximum score of 24 in response to the female conspecific. Nevertheless, following saline injections, the average response to urine alone was only 64% of the response obtained to the female.

After being given an ip injection of sodium salicylate, male vocalizations decreased even when the male was tested with a female conspecific. This decrease may be a result of cooling the hypothalamus (Blumberg & Moltz, 1987). The amount of vocalizations made in response to female urine alone was also reduced. Thus, the data obtained in this experiment are consistent with those obtained in rats by Blumberg and Moltz. In both studies treatment with sodium salicylate reduced male USV (70-kHz courtship calls in mice, and 22 kHz post-ejaculatory calls of the rat).

In their experiment, Blumberg & Moltz (1987) did not report any effects of sodium salicylate on the mount latency of their rats. But they did report that after treatment with sodium salicylate, two of the six animals they tested failed to achieve an intromission in their first test with a receptive female.

In the present study, mice appeared to be much more sensitive to the effects of sodium salicylate on mounting behavior. All of the mice did not attempt to amount the female in the period allowed. It is not clear if the proportion of animals that failed to mount would have been lower if they had been allowed more time to do so. Males took longer to mount the female after treatment with sodium salicylate. Both the low and the high dose had similar effects; none of the subjects in either dose group mounted a female during treatment with sodium salicylate.

However, there was an interaction between dose and drug treatments. The longest mount latencies after treatment with saline were observed in animals receiving the high drug dose. It is interesting that the males in this group who had the longest latencies were all tested first following treatment with sodium salicylate and then following saline treatment. Males in the high dose condition that had been tested in the drug condition first, had longer mount latencies (112.75 ± 3.82) when tested with saline, than animals in the low dose group tested in the same order (83.86 ± 8.38). Thus, the interaction effect could be due to a prolonged influence of sodium salicylate at the highest dose that was employed (150mg/kg). It is possible that this prolonged effect may be due to prolonged reductions in brain temperature, or perhaps it is due to drug induced nausea.

To distinguish between these two possibilities, mice could be tested after infusing very small quantities of a sodium salicylate solution directly into the hypothalamus and presenting the female before the drug has a chance to diffuse throughout the body. Alternatively, a lower and wider range of sodium salicylate doses could be administered by ip injection. At lower doses than those employed in this study, sodium salicylate may still reduce USV but may be less likely to induce nausea.

In summary, our findings support the hypothesis that USV are a result of increased hypothalamic temperature (Blumberg & Moltz, 1987). Serotonergic anxiolytics also reduce USV (Miczek, Tornatzky, & Vivian, 1991). In addition

to their anxiolytic effects, these drugs also affect temperature regulation. Further studies need to be conducted to see how anxiolytic and antipyretic agents reduce USV and to distinguish the antipyretic mechanisms from the anxiolytic ones.

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